

Nanocellulose Reinforced Chitosan Composite Films as Affected by Nanofiller Loading and Plasticizer Content

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ABSTRACT: Chitosan is a biopolymer obtained by N-deacetylation of chitin, produced from shellfish waste, which may be employed to elaborate edible films or coatings to enhance shelf life of food products. This study was conducted to evaluate the effect of different concentrations of nanofiller (cellulose nanofibers, CNF) and plasticizer (glycerol) on tensile properties (tensile strength—TS, elongation at break—EB, and Young's modulus—YM), water vapor permeability (WVP), and glass transition temperature (T_g) of chitosan edible films, and to establish a formulation to optimize their properties. The experiment was conducted according to a central composite design, with 2 variables: CNF (0 to 20 g/100 g) and glycerol (0 to 30 g/100 g) concentrations in the film (on a dry basis), which was produced by the so-called casting technique. Most responses (except by EB) were favored by high CNF concentrations and low glycerol contents. The optimization was based on maximizing TS, YM, and T_g , and decreasing WVP, while maintaining a minimum acceptable EB of 10%. The optimum conditions were defined as: glycerol concentration, 18 g/100 g; and CNF concentration, 15 g/100 g. AFM imaging of films suggested good dispersion of the CNF and good CNF-matrix interactions, which explains the good performance of the nanocomposite films.

Practical Application: Chitosan is a biodegradable polymer which may be used to elaborate edible films or coatings to enhance shelf life of foods. This study demonstrates how cellulose nanofibers (CNF) can improve the mechanical and water vapor barrier properties of chitosan films. A nanocomposite film with 15% CNF and plasticized with 18% glycerol was comparable to some synthetic polymers in terms of strength and stiffness, but with poorer elongation and water vapor barrier, indicating that they can be used for applications that do not require high flexibility and/or water vapor barrier. The more important advantage of such films when compared to synthetic polymer films is their environmentally friendly properties.

Keywords: edible films, nanocomposite, nanotechnology

Introduction

Chitosan [β -(1,4)-2-amino-2-deoxy-D-glucopyranose] is a biopolymer obtained by N-deacetylation of chitin, which is the 2nd most abundant polysaccharide on nature after cellulose (Arvanitoyannis and others 1998). It can be produced from shellfish waste, and is composed primarily of glucosamine or 2-amino-2-deoxy-D-glucose units (Sandford 1989). Chitosan forms clean, tough, and flexible films with good oxygen barrier (Jeon and others 2002), which may be employed as packaging, particularly as an edible film or coating (Bangyekan and others 2006), enhancing shelf life of food products (Durango and others 2006; Campaniello and others 2008). Chitosan can form a semipermeable coating that can modify the internal atmosphere, thereby decreasing

transpiration rates in produce (Nisperos-Carriedo 1994). Chitosan films are brittle (Suyatma and others 2004), thus a plasticizer is needed to increase film flexibility. Plasticizers like glycerol may improve the processability of chitosan, and the mechanical properties of chitosan films, according to Suyatma and others (2005), who reported that a glycerol concentration of 20% (w/w) was sufficient to improve flexibility of chitosan films. Di Gioia and Guilbert (1999) presented several theories that have been proposed to explain mechanisms of plasticization action. The lubrication theory postulates that plasticizers, by interspersing themselves, act as internal lubricants by reducing frictional forces between polymer chains. According to the gel theory, the rigidity of polymer comes from 3-dimensional structures, and plasticizers act by breaking polymer-polymer interactions (like hydrogen bonds and van der Waals forces) and forming secondary bonds to polymer chains, causing adjacent chains to move apart and so reducing film rigidity and increasing flexibility. The most effective plasticizers are similar to the polymer structure, so hydrophilic plasticizers such as polyols are best suited to polysaccharide films (Sothornvit and Krochta 2005). Due to the stiffness of the backbone and the molecule configuration, the glass transition (T_g) of chitosan was attributed by Quijada-Garrido and others (2008) to torsional oscillations between 2 glucosamine rings across glucosidic oxygen and a cooperative reordering of hydrogen bonds.

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However, the use of chitosan and other biopolymers has been limited because of their usually poor mechanical and barrier properties when compared to those of synthetic polymers. The addition of reinforcing fillers to biopolymers has proven to be effective in enhancing their thermal, mechanical, and barrier properties. The smaller the filler particles, the better is the interaction filler matrix (Ludueña and others 2007), and usually the better is the cost price efficiency (Sorrentino and others 2007). Fillers with at least 1 dimension in the nanometric range are called nanoparticles (nanoreinforcements), and their composites with polymers are nanocomposites (Alexandre and Dubois 2000). A uniform dispersion of nanoparticles leads to a very large matrix/filler interfacial area, which changes the molecular mobility, the relaxation behavior, and the consequent thermal and mechanical properties of the material (Vaia and Wagner 2004).

Cellulose nanofibers are very interesting nanomaterials for production of cheap, lightweight, and very strong nanocomposites (Podsiadlo and others 2005). The cellulose chains are synthesized to form nanofibers, which are elongated bundles of molecules stabilized through hydrogen bonding. Nanofibers have nanosized diameters (2 to 20 nm), and lengths ranging from a few hundred nanometers up to a few micrometers (Azizi Samir and others 2005). Cellulose nanofibers are recognized as being more effective than their micro-sized counterparts to reinforce polymers due to interactions between the nanosized elements that form a percolated network connected by hydrogen bonds, provided there is a good dispersion of the nanofibers in the matrix (Anglès and Dufresne 2001; Nakagaito and others 2009).

This study was conducted to evaluate the effect of different concentrations of cellulose nanofibers (added as nanoreinforcement) and glycerol (plasticizer) on tensile properties, water vapor permeability, and glass transition temperature of chitosan edible films, and to establish an optimum formulation, that is, the formulation that provided the best possible combinations of properties related to performance of the material as food packaging.

Materials and Methods

The cellulose nanofibers (Avicel[®] PH) were provided by FMC BioPolymer (Philadelphia, Pa., U.S.A.). To measure the average dimensions (diameter and length) of the nanofibers, an aliquot of the cellulose nanofiber (CNF) solution was mixed with an equal volume of 2% uranyl acetate (UA). A 10- μ L drop of the UA–CNF mixture was dispensed onto a 400 mesh copper grid, allowed to stand for 30 to 60 s, and the excess fluid was wicked off with Whatman nr 1 filter paper (Whatman Ltd., Maidstone, U.K.). The grid was air dried and viewed in a CM12 scanning-transmission electron microscope (STEM, FEI Co., Inc., Hillsboro, Oreg., U.S.A.) operating in the bright field mode at 80 kV. Digital images were captured with the STEM's associated XR41 CCD camera system (AMT, Danvers, Mass., U.S.A.). Fiber lengths and widths were measured directly from transmission electron micrographs using Image Pro Plus 6.3 (Media Cybernetics, Inc., Bethesda, Md., U.S.A.). Data were collected and analyzed using Microsoft Excel 2003.

The experiment was conducted according to a central composite design (Table 1), with 2 variables: cellulose nanofibers (0 to 20 g/100 g) and glycerol (0 to 30 g/100 g) concentrations in the film (on a dry basis). A dilute chitosan solution was made by preparing a 3% chitosan (MW = 71.3 kDa, degree of deacetylation 94%, purchased from Polymar Ciência e Nutrição S/A, Fortaleza, Brazil) in 1.5% acetic acid solution. Cellulose nanofibers (CNF) and glycerol were added to the chitosan solution, and the dispersions were homogenized at 4500 rpm for 30 min, by using a Polytron PT 3000 (Brinkmann Instruments, Westbury, N.Y., U.S.A.). The film-forming

dispersions were submitted to vacuum to remove bubbles, and films were cast on leveled 29 \times 29 cm square plates (50 g/plate) and allowed to dry for 16 h at 22 °C and 42% relative humidity (RH). Samples of the dried films were cut and peeled from the casting surface, and stored under refrigeration until analyses.

Film thicknesses were measured with a micrometer IP 65 (Mitutoyo Manufacturing, Tokyo, Japan) to the nearest 1 μ m, at 8 random positions around the film for samples designed for water vapor permeability (WVP) and tensile tests.

Tensile properties (tensile strength—TS, elongation at break—EB, and Young's modulus—YM) were measured according to standard method D882-97 (ASTM 1997), by using an Instron Model 55R4502 Universal Testing Machine (Instron, Canton, Mass., U.S.A.) with a 100 N load cell. The detailed methods for both WVP and tensile tests were described by Rojas-Graü and others (2007). The gravimetric Modified Cup Method (McHugh and others 1993) based on standard method E96-80 (ASTM 1989) was used to determine WVP. The glass transition temperature (T_g) of the nanocomposite films was measured by differential scanning calorimetry (DSC) by using an mDSC 2910 (TA Instruments, New Castle, Del., U.S.A.). The DSC profiles were run from 30 to 200 °C, at a heating rate of 10 °C/min. Eight specimens of mango puree edible film (MPEF) from each experimental run were evaluated for measuring tensile properties and WVP. For T_g measurements, 4 specimens were evaluated.

The results were analyzed by using the software Minitab[®] 15 (Minitab Inc., State College, Pa., U.S.A.). The models generated to represent the responses were evaluated in terms of their F ratio and R^2 coefficient. The influence of the variables on the responses was evaluated by studying the contour plots generated from the models. To establish an experimental region that could provide satisfactory values of all responses, the "overlaid contour plot" function of the software was used. Lower limits were defined for the responses to be maximized (TS, EB, YM, and T_g), and an upper limit for WVP (which was to be minimized). Within the intersection region of the limits defined, an experimental point was chosen and defined as representing the optimum conditions. Such conditions were used to elaborate a validation run (in 5 replications), to compare its actual (experimental) results with the corresponding predicted results (by the regressions) and verify the accuracy of the models.

Atomic force microscopy (AFM) images were taken from 2 chitosan films, one produced from the optimum conditions, and the other with the same glycerol concentration but without CNF. The images were captured by using an Ntegra microscope (NT-MDT, Russia) under contact mode in air with silicon probes (rectangular

Table 1 – Glycerol and CNF concentrations (on a dry basis) for each run.

Run	Glycerol		CNF	
	Coded	Uncoded (g/100 g)	Coded	Uncoded (g/100 g)
Control	–1.41	0	–1.41	0
1	–1	4.36	–1	2.91
2	1	25.64	–1	2.91
3	–1	4.36	1	17.09
4	1	25.64	1	17.09
5	–1.41	0	0	10
6	1.41	30	0	10
7	0	15	–1.41	0
8	0	15	1.41	20
9	0	15	0	10
10	0	15	0	10
11	0	15	0	10

cantilevers with nominal spring constant of 0.01 to 0.08 N/m and nominal tip curvature radius of 10 nm).

Results and Discussion

Figure 1 presents a TEM image of the CNF, whose average length and diameter were, respectively, 98.1 ± 4.7 and 8.0 ± 3.4 nm. Although the aspect ratio of the fibers (about 12.3) was lower than the values reported in other studies (Lima and Borsali 2004; Rodriguez and others 2006), it was still higher than 10, which was mentioned by Mutjé and others (2007) as being the minimum aspect ratio required for a good stress transfer from the matrix to the fibers to promote a significant reinforcement.

Films with an average thickness of 0.029 mm were produced. Table 2 presents the experimental responses for each run, and Table 3 presents the regression coefficients of the models (in coded values). Full quadratic models were adopted for all responses except for T_g , for which a linear model was fitted better. Although 2 of the models (those for EB and WVP) have presented significant lack of fit ($P < 0.05$), all regressions were highly significant ($P < 0.01$), and the R^2 coefficients were satisfactory (above 94%), indicating that the models could be considered adequate for representing the responses.

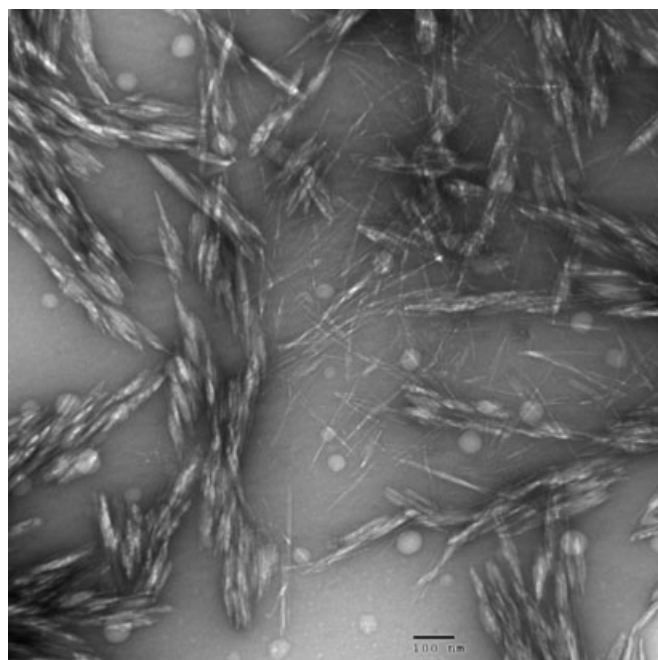


Figure 1 – Cellulose nanofibers visualized by TEM.

Table 2 – Average experimental responses for each experimental run on chitosan films.

Run	TS (MPa)	EB (%)	YM (MPa)	WVP (g mm/kPa/day/m ²)	T_g (°C)
Control	47.68	7.89	1304.32	17.29	130.1
1	47.51	6.28	1462	15.82	128.7
2	33.03	26.97	817.95	18.10	125.3
3	55.64	5.42	1632.83	12.91	136.7
4	37.62	24.28	970.48	15.82	133.3
5	57.45	7.63	1627.67	14.11	135.9
6	28.01	34.60	734.23	19.63	126.9
7	45.97	15.63	1272.33	17.88	127
8	53.73	7.91	1428	12.22	138.1
9	52.34	8.20	1436.83	12.84	131
10	52.10	7.69	1379.58	12.98	130.2
11	55.53	8.03	1424.03	12.91	132.5

TS = tensile strength; EB = elongation at break; YM = Young's modulus; WVP = water vapor permeability; T_g = glass transition temperature.

The contour plots for the physical properties of the nanocomposite films (Figure 2) indicate that CNF increased the strength and the modulus of the films, but decreased their elongation. Similar mechanical performances were reported for the addition of cellulose nanofibers to other biopolymers (Tang and Liu 2008; Suryanegara and others 2009; Wan and others 2009). Similarly to CNF, Xu and others (2006) observed that the nanoclay montmorillonite increased strength and decreased elongation of chitosan films. The increased strength and modulus of the nanocomposites suggest the formation of an exfoliated nanocomposite with uniform dispersion of the fibers within the polymer matrix, as well as and good fiber-polymer adhesion interactions (Xu and others 2006; Gardner and others 2008; Wan and others 2009). Differently from this study, some researchers (Dogan and McHugh 2007; Tang and Liu 2008) observed that CNF did not significantly affect elongation of films, while other studies (Wu and others 2007; Nakagaito and others 2009) reported that even the elongation was improved by CNF addition. This is possibly explained by a better adhesion of CNF to the matrixes used in those studies than in the present one. Glycerol, as expected, presented the opposite effects, that is to say, it improved elongation but impaired strength and stiffness, as previously described for other biopolymer films (Arvanitoyannis and others 1998; Bangyekan and others 2006; Talja and others 2007). The addition of plasticizers in most biopolymer films is required to overcome film brittleness caused by extensive intermolecular forces. Plasticizers reduce such forces, thereby improving flexibility and extensibility of the films (Forsell and others 2002). On the other hand, they decrease crystallinity of biopolymer films, leading to a significant decrease in the film strength and modulus (Bangyekan and others 2006).

WVP was decreased by increasing CNF concentrations (Figure 2). According to Lagaron and others (2004), the presence of impermeable crystalline fibers increases path tortuosity leading to slower diffusion processes and, hence, to lower permeability. Casariego and others (2009) reported that the WVP of chitosan films was also decreased by addition of clay micro/nanoparticles. On the other hand, the water vapor barrier in this study was impaired by increasing glycerol concentration, which was expected from a hygroscopic plasticizer like glycerol, and is consistent with previous results (Bertuzzi and others 2007; Talja and others 2007). Since plasticizers extend, dilute, and soften the polymer structure, the chain mobility is increased and diffusion coefficients for gas or water is markedly increased (Guilbert 1986).

T_g of the films were increased by CNF (Figure 2), corroborating other reports of T_g increasing effects from adding cellulose nanofibers to biopolymer films (Anglès and Dufresne 2000; Alemдар and Sain 2008). On the other hand, Azizi Samir and others (2004) did not observe changes in T_g of poly(oxyethylene)

by addition of cellulose nanocrystals. T_g was decreased by glycerol, because of its plasticizing effect, corroborating reports by Quijada-Garrido and others (2008). Plasticization occurs in the higher molecular mobility (amorphous) region of the polymer, where they interrupt hydrogen bonding along the polymer chains, increasing T_g (Sothornvit and Krochta 2005). The effects of the variables on T_g of chitosan films (that is, its depression by glycerol and increase by CNF) were illustrated in the DSC profiles (Figure 3).

Most responses (except by EB) were favored by high CNF concentrations and low glycerol contents. However, EB was impaired by such conditions. The overlaid contour plot (Figure 4) was created according to the following criteria: TS \geq 50 MPa; YM \geq

1300 MPa; EB \geq 10%; $T_g \geq$ 130 °C; WVP \leq 13 g mm/kPa/day/m². The optimum conditions, defined within the dark area in Figure 3, were thus identified as being: glycerol concentration, 18 g/100 g; and CNF concentration, 15 g/100 g (both added to chitosan on a dry basis). The observed responses at such conditions were satisfactorily near the predicted ones (Table 4). When compared to the control (Table 2), the film production under optimum conditions resulted in improvements in mechanical properties (especially elongation, whose increase was about 30%) and water vapor permeability (which decreased in about 27%). Film strength and modulus were within the ranges reported for some synthetic polymers and biopolymers, or even better than those (Table 4), except for

Table 3 – Estimated regression coefficients for the responses (coded values).

Term	TS	EB	YM	WVP	T_g	
Constant	53.32	7.97	1413.48	12.91	131.42	
[GLY]	-9.27	9.71	-321.24	1.62	-2.44	
[CNF]	2.96	-1.81	67.94	-1.65	3.96	
[GLY] ²	-6.01	6.39	-127.45	1.91	—	
[CNF] ²	-2.45	1.72	-42.84	0.99	—	
[GLY][CNF]	-0.89	-0.46	-4.57	0.16	—	
R^2 (%)	96.6	99.2	99.2	96.9	94.3	
Regression	<i>F</i> ratio	28.21	124.05	129.49	31.54	66.03
	<i>P</i>	<0.01	<0.01	<0.01	<0.01	<0.01
Lack of fit	<i>F</i> ratio	2.45	39.68	2.05	131.05	0.95
	<i>P</i>	0.30	0.03	0.34	<0.01	0.59

Terms in bold for the regression were significant ($P < 0.05$). TS = tensile strength; EB = elongation at break; YM = Young's modulus; WVP = water vapor permeability; T_g = glass transition temperature; [GLY] = glycerol concentration; [CNF] = cellulose nanofibers concentration.

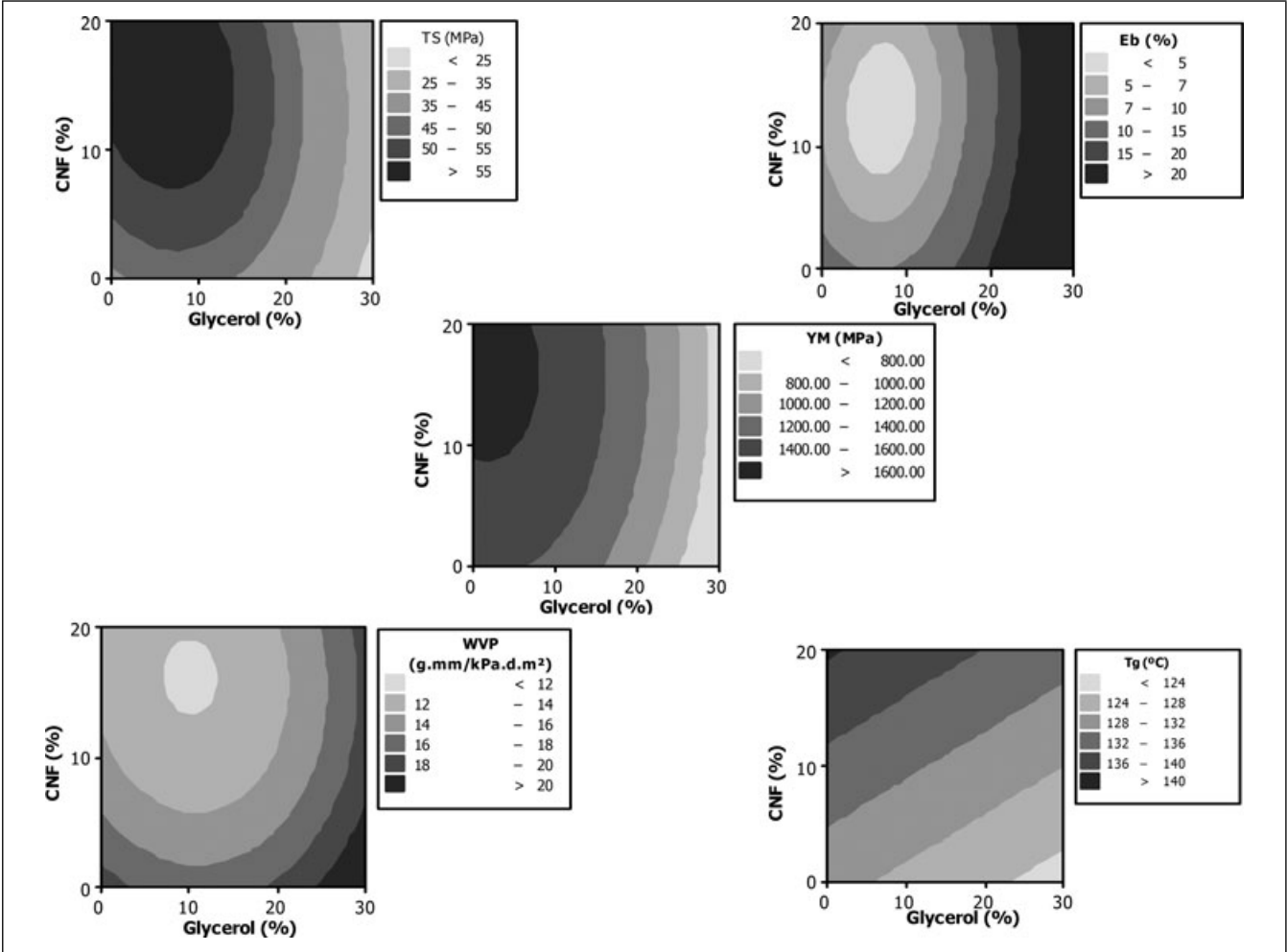


Figure 2 – Physical properties of chitosan–CNF nanocomposite films.

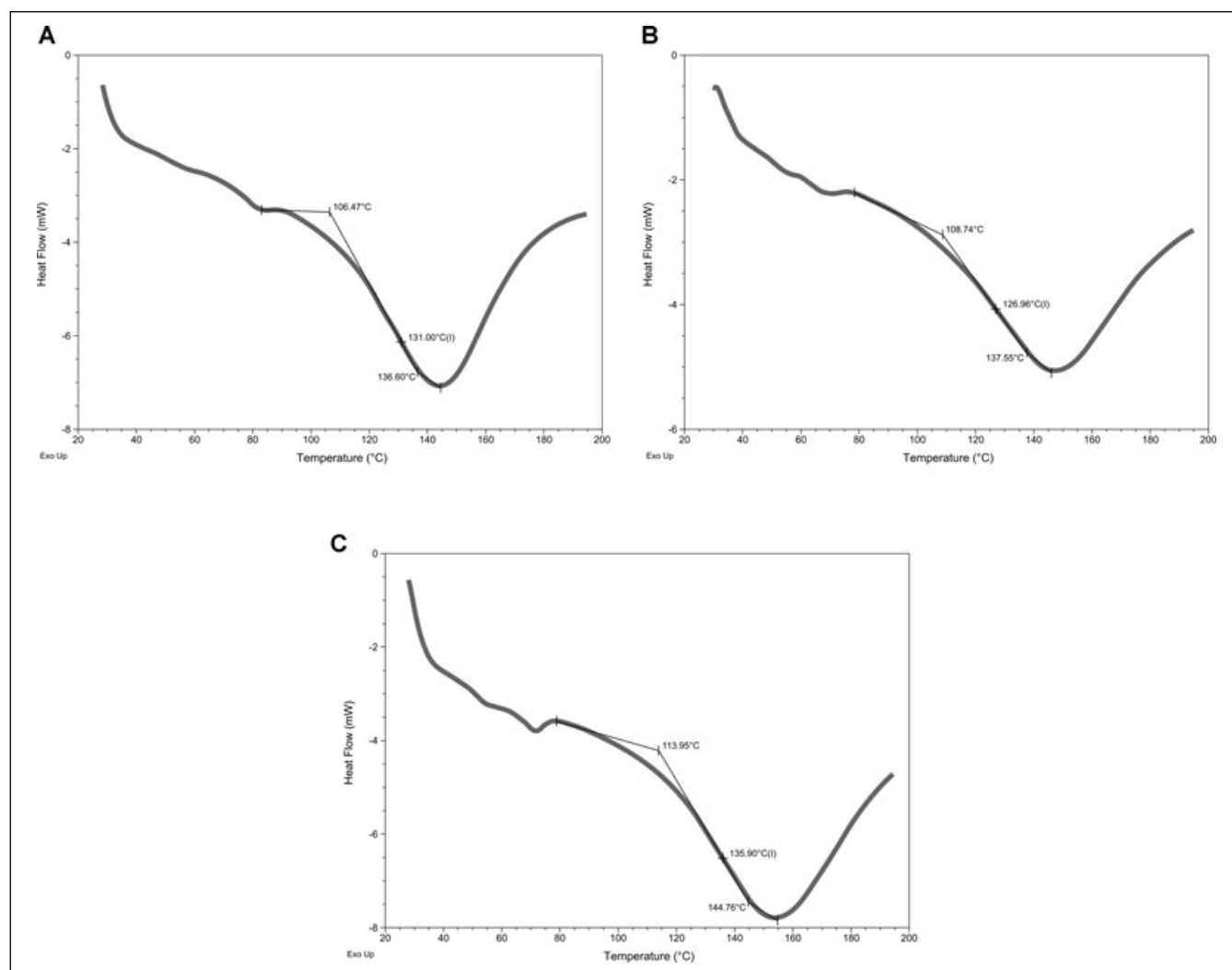


Figure 3 – Typical DSC profiles from films: (A) control (without glycerol and CNF); (B) with glycerol (15 g/100 g) and without CNF; (C) with CNF (10 g/100 g) and without glycerol.

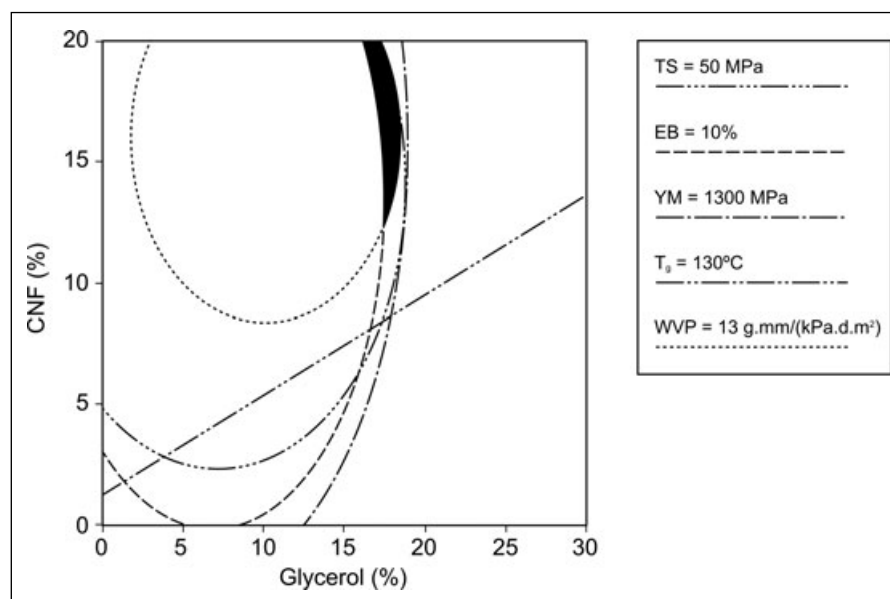
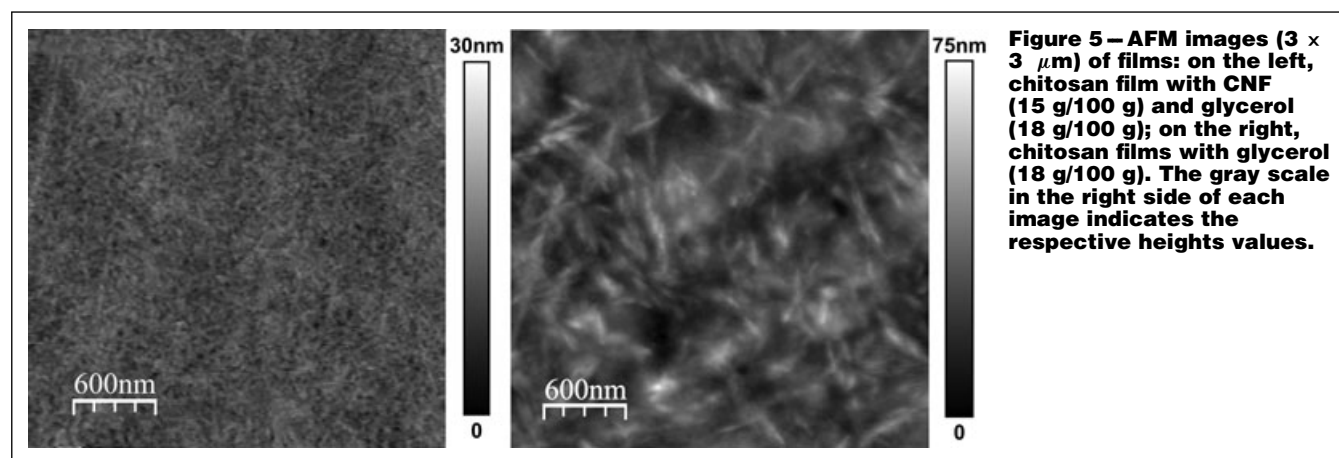


Figure 4 – Overlaid contour plots of the responses. The dark area is the intersection region of the limits, from which the “optimum conditions” were defined.

Table 4 – Predicted and actual (experimental) responses from the optimum conditions of CNF/chitosan film elaboration, compared to values previously reported.

Materials	TS (MPa)	EB (%)	YM (MPa)	WVP (g mm/kPa/day/m ²)	T _g (°C)
CNF/CS ^a	50.92	10.72	1338.13	12.89	133.5
CNF/CS ^b	52.7 (3.5)	10.3 (0.5)	1367.9 (79.4)	12.6 (0.6)	129.2 (6.6)
LDPE	8 to 31 ^d	125 to 675 ^c	200 to 500 ^d	0.066 to 0.099 ^e	–120 ^d
PP	31 to 43 ^d	100 to 600 ^c	1140 to 1550 ^e	0.06 ^e	–10 ^d
PS	14 to 70 ^e	1.0 to 2.3 ^c	2280 to 3280 ^e	0.46 to 0.66 ^e	74 to 105 ^e
PVC	10 to 55 ^c	200 to 450 ^c	3 to 21 ^c	0.03 to 0.20 ^e	75 to 105 ^d
Alginate	18 to 49 ^f	6.5 to 13 ^f	122 to 480 ^f	7 to 14 ^g	164.4 ^h
WPI/SDS	3 to 9 ⁱ	10 to 65 ⁱ	100 to 550 ^j	65 to 96 ⁱ	—
Gelatin	47 to 85 ^j	3 to 8 ^j	1978 to 2245 ^j	17 to 45 ^j	41 to 82 ^k
Pea starch	3 to 45 ^l	4 to 94 ^l	41 to 1584 ^l	46 to 66 ^l	–85 to (–4) ^l
HPMC	28.3 ^m	8.1 ^m	900 ^m	19.06 ^m	—

TS = tensile strength; EB = elongation at break; YM = Young's modulus; WVP = water vapor permeability; T_g = glass transition temperature. CNF/CS = cellulose nanofiber/chitosan nanocomposite film (this study), optimum formulation, ^apredicted and ^bexperimental values (standard deviations between parentheses). LDPE = low-density polyethylene; PP = polypropylene; PS = polystyrene; PVC = poly(vinyl chloride); WPI/SDS = whey protein isolate and sodium dodecyl sulfate; HPMC = hydroxypropyl methylcellulose. ^{c–k}Approximate values/ranges from data compiled in previous studies: ^cShackelford and Alexander (2001), ^dSelke (2003), ^eHernandez (1997), ^fLima and others (2007), ^gZaciti and Kieckbusch (2006), ^hMcPhillips and others (1999), ⁱFairley and others (1996), ^jChiou and others (2008), ^kDe Moura and others (2009), ^lZhang and Han (2006), ^mVanin and others (2005).



gelatin films, which were reported to have better modulus values (Chiou and others 2008). On the other hand, the elongation was poor when compared to synthetic materials but polystyrene (PS), but within the ranges reported for other biopolymers, and better than values reported for gelatin films. WVP of the films was much higher than those reported for synthetic polymers, but similar or lower than values reported for other biopolymer films. Anyway, the water vapor barrier could be improved by addition of some lipid component to the film formulation. The T_g of the film was higher than those reported for synthetic polymers and other biopolymers, indicating the high brittleness of the film, corroborating its poor elongation.

Figure 5 presents typical AFM topography images of films produced from chitosan added with CNF and glycerol (left), and only with glycerol. The addition of CNF increased the surface root mean square roughness of film from 1.6 to 8.3 nm. The rod-shaped structures observed on the film with CNF have no specific orientation, which suggests an exfoliated nanocomposite. The mean length of rods is compatible with the length of CNF, but its mean width is larger (approximately 20 nm). This difference could be caused by AFM tip dilatation artifact in the images, which increases the real CNF width, or is an indication of the presence of nanometric CNF bundles (of 2 nanofibers each) in the sample. There were apparently good interactions between nanofibers and chitosan, which can explain the good performance of the nanocomposite films investigated.

Conclusions

Mechanical and water vapor barrier properties of chitosan films were improved by addition of cellulose nanofibers. A nanocomposite film with 15% cellulose nanofibers and plasticized with 18% glycerol was comparable to synthetic polymers in terms of strength and stiffness, but their elongation and water vapor barrier were poorer, indicating that the film may be used only for applications that do not require a great flexibility and/or water vapor barrier.

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